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at 120 and 90 kD, respectively (Fig. 3B) (*12*). This result indicated that both motifs are expressed as products on the cell surface that encode the binding site for HBGF. Because the sizes of the extracellular structures predicted from the aa sequence are 44 and 34 kD, respectively, the two structures may contain 60 and 40 kD of carbohydrate. In contrast to constructions coding for the α and β extracellular motifs, the comparable γ motif construction caused no increase in transfected cell surface HBGF binding that could be detected by either Scatchard analysis or ligand affinity cross-linking. Stability, activity, and cellular location of γ translation products is under investigation.

To determine HBGF binding activity of full-length receptor isoforms, in COS cells we expressed constructs that contained α or β extracellular domains fused to each of the two intracellular domain motifs (Fig. 3, C and D). Expression of full-length constructs caused a 5- to 15-fold increase in specific HBGF-1 (Fig. 3A) and HBGF-2 binding sites per cell, with an apparent K_d of 100 to 500 pM (*11*). Constructs coding for the α motif yielded [^{125}I]HBGF-1–labeled expression products that were about 30 kD larger than constructs coding for the β motif. Finally, constructs coding for the b2 intracellular domain exhibited [^{125}I]HBGF-labeled expression products that were about 20 kD smaller than constructs coding for the a1 intracellular domain (*13, 14*).

The three distinct structural domains that combine to form HBGF receptor isoforms are likely to affect ligand binding, oligomerization, cellular location, metabolism, and signal transduction (*15*). The α and β extracellular motifs appear to differentially oligomerize (*12*), and ligand binding may be affected by the intracellular domain motif with which it is combined (Fig. 3, C and D) (*12, 13*). The cDNA that encodes the γ motif may result in an intracellular form of the receptor. The a- and b-type juxtamembrane motifs contain different candidate phosphorylation sites for a Ser-Thr protein kinase. Juxtamembrane phosphorylation sites have been implicated in alteration of ligand affinity, kinase activity, and internalization (down-regulation) of tyrosine kinase receptors (*15, 16*). The two COOH-terminal motifs may differ in tyrosine kinase activity, in interaction with intracellular substrates, and as substrates for tyrosine kinases in the COOH-terminus (*15*).

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7. The 1.8-, 1.1-, and 0.6-kb clones, identified by hybridization with an oligonucleotide (01) in the 5' sequence of a1 and a2 cDNAs (Fig. 1B), overlapped a1 and b2 cDNAs by 178, 53, and 96 nucleotides, respectively. The 1.8-kb cDNA, when ligated to a1 or b2, represents a near full-length 4.2-kb mRNA that encoded a three–IgG-like loop extracellular domain (α) (Fig. 1). The 1.1- and 0.6-kb cDNAs exhibited a deletion of 267 bp that encoded the 89-residue outermost IgG-like loop (Fig. 1). The 306-bp preceding the common translational initiation site and secretory signal sequence in the 1.1- and 1.8-kb cDNAs were identical. The remaining 5' noncoding sequences were unique.

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9. The P1a primer sequence is indicated in Fig. 1B. The sequence of other primers was as follows: P2b, GCTTGCCCAGATCTCCAG; P2c, CCTGCTCTGCTGGAGAGGAAC; 01, GACCTTGTAGCCTCC; P2d, CCCATTCACCTCGATGTGCTT; P3a, TTGGCGGGTAACTCTATCGGACTC; P1b GTTACCCGCCAAGCACGTATAC; P6, TATATGAATTCGTGCACAGCCATCTGGCTGTGGAA; P3b, AGCAGACACTGTTAC; P5b, CGGCCATCACGGCTCTCCTCCAGTGGCG; P2a, GGGCCTCCGGGCCTGCAGGTACTCCG; P5c, GAGCCTTCAGGTTCCACA; and P5a, CGAAAGACCACACATCA.

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11. Cells transfected with the extracellular domain constructions exhibited a higher number of binding sites per cell with higher apparent K_d (lower affinity) than cells transfected with full-length constructions. Otherwise, no consistent difference in apparent K_d for HBGF binding has been demonstrated among receptor isoforms by Scatchard analysis. Untransfected COS cells displayed about 4000 sites per cell with apparent K_d of 100 pM. Since COS cells express uncharacterized receptor isoforms, the apparent K_d is likely a composite of host cell and transfected receptor species.
12. Although cross-linking artifact cannot be eliminated, higher molecular size species of ligand-receptor complexes may indicate self-oligomerization or activation and association of transfected products with host cell receptor species. Oligomeric bands are more apparent in cells transfected with β constructs, independent of COOH-terminus (Fig. 3) (J. Hou *et al.*, unpublished data).
13. Constructions that coded for the a1 intracellular domain exhibited more intensely HBGF-labeled species than the b2 motifs, as evident in Fig. 3, C and D. Separate experiments with b1 and a2 constructions in permanently transfected cells indicated that the reduced ligand-binding is due predominately to the b juxtamembrane motif and to a lesser extent, the type 2 COOH-terminal motif, independent of extracellular domain (J. Hou *et al.*, in preparation).
14. Untransfected COS cells exhibited a single HBGF labeled band at 150 kD. HepG2 cells displayed labeled bands of 120, 150, and 280 kD, the most intense of which was 120 kD.

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17. We thank E. Mansson, S. Harris, and S. Goodrich for advice and synthesis of oligonucleotides, M. Mueckler for the HepG2 λgt11 phage library, E. Shi for assistance in [^{125}I]HBGF labeling, J. Huang and F. Wang for assistance in analysis of cDNA clones, and D. Fast and M. Kan for assistance in culture of HepG2 cells. Supported by grants from the U.S. National Institutes of Digestive and Kidney Diseases and the National Cancer Institute.

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Inability of Malaria Vaccine to Induce Antibodies to a Protective Epitope Within Its Sequence

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***Saimiri* monkeys immunized with a recombinant protein containing 20 copies of the nine amino acid repeat of the *Plasmodium vivax* circumsporozoite (CS) protein developed high concentrations of antibodies to the repeat sequence and to sporozoites, but were not protected against challenge. After intravenous injection of an immunoglobulin G3 monoclonal antibody (NVS3) against irradiated *P. vivax* sporozoites, four of six monkeys were protected against sporozoite-induced malaria, and the remaining two animals took significantly longer to become parasitemic. Epitope mapping demonstrated that NVS3 recognizes only four (AGDR) of the nine amino acids within the repeat region of the *P. vivax* CS protein. The monkeys immunized with $(\mathrm{DRA}^{A}_{D}\mathrm{GQPAG})_{20}$ did not produce antibodies to the protective epitope AGDR. Thus, determination of the fine specificity of protective immune responses may be critical to the construction of successful subunit vaccines.**

DURING RECENT YEARS THERE HAS been considerable effort to produce vaccines designed to induce protective antibodies against repetitive sequences on the CS protein of *Plasmodium*, which causes human malaria. These efforts have been, in large part, based on the observation that passive transfer of monoclonal antibodies against the CS protein of rodent parasites *Plasmodium berghei* (*1, 2*) and *P. yoelii* (*3*) protects against challenge with sporozoites. Incubation of *P. falciparum* or *P. vivax* sporozoites with Fab fragments of monoclo-

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nal antibodies to their respective CS proteins reduced the subsequent infectivity of the sporozoites to chimpanzees (*4*). However, it has never been definitively established in man that circulating antibodies to the sporozoites of *Plasmodium* can prevent infection.

The levels of protection have been disappointing in humans immunized with *P. falciparum* CS protein vaccines (*5, 6*) and in *Saimiri* monkeys immunized with two subunit *P. vivax* vaccines (*7*). The unprotected *Saimiri* monkeys all developed high concentrations of antibodies to the CS protein, raising the question of whether the lack of protection was because the vaccine-induced antibodies were directed against the wrong epitopes or because humoral immunity is insufficient to neutralize *P. vivax* sporozoites. The current studies were undertaken to determine if circulating antibodies to the *P. vivax* CS protein could protect *Saimiri* monkeys against sporozoite-induced malaria, and to define the target epitope of any such protective immunity.

Six monkeys (*Saimiri sciureus boliviensis*) (*8*) were each inoculated intravenously with 2 mg of NVS3, an IgG3 isotype monoclonal antibody to *P. vivax* sporozoites (*9*), 1 hour before receiving an intravenous 10^4 *P. vivax* sporozoite challenge (*10*). An additional six control monkeys received 2 mg of a monoclonal antibody directed against *Trypanosoma brucei rhodesiense* (anti-trypanosoma antibody) before sporozoite challenge. Four of the six monkeys inoculated with NVS3 were fully protected against blood stage disease; the remaining two developed patent parasitemias after 31 and 40 days (Table 1). The two unprotected monkeys that received NVS3 had longer prepatency periods than the monkeys receiving the anti-trypanosomal antibody ($P < 0.01$) and longer than nine uninjected controls ($P < 0.005$). To determine the exact epitope of NVS3, we used an epitope-scanning technique (*11*) to synthesize 137 octapeptides based on the nonapeptide repeat sequences of the following four strains of *P. vivax*: Belem (*12*), Sal 1 (*13*), North Korean (*14*), and VS 210 (*15*). By enzyme-linked immunosorbent assay (ELISA), NVS3 reacted only with the tetrapeptide AGDR (alanine–glycine–aspartic acid–arginine) (Fig. 1). Octapeptides containing subsets of AGDR (AGD and GDR) were not reactive. No correlation between reactivity and the location of the tetrapeptide within the octapeptide was noted (Fig. 2). We then synthesized the eight-residue peptide $(AGDR)_2$ (*16*). An immunofluorescent antibody technique (IFAT) showed that NVS3 binds to *P. vivax* sporozoites (but not to *P. yoelii* sporozoites) and that this binding could be specifically

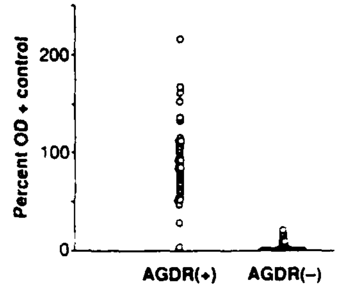

Fig. 1. Antibody-octapeptide reactivity of peptides containing the entire AGDR sequence ($AGDR^+$) ($n = 50$) and those with part or none ($AGDR^-$) ($n = 87$) is plotted against percent of the optical density of the positive control. The n values are the total number of octapeptides containing AGDR ($n = 50$) and not containing AGDR ($n = 87$). The positive control optical density was obtained with a monoclonal antibody to PLAQ. These data were developed by designing a hypothetical peptide containing the repeat regions of the CS proteins of four strains of *P. vivax*. The sequence of the peptide is as follows: GDRADGQPAGDRADGQPAGDRA-DGQAAGNGAGGQPAGDRAAGQPAGDGA-AGQPAGDRADGQPAGDRAAGQPAGDRA-DGQPAGDRADGQAAGNGAGGQAAGNGAGGQPAGDRAAGQPAGDRAAGQPAGDRAAG-QAAGNGAGGQAA. We synthesized 137 sequential octapeptide subsets of this 144–amino acid peptide. The octapeptides were synthesized as per Geysen (*11*) on the tips of polypropylene pins set in 96-pin blocks (Cambridge Research Biochemicals, Valley Stream, New York). Octapeptide n = amino acid n through amino acid $n + 7$. The syntheses were carried out in 96-well plates, thereby allowing each pin to hold a different amino acid sequence. Conventional Fmoc (fluorenyl methoxycarbonyl) solid-phase methods were used to complete the syntheses. The tetrapeptides PLAQ (and monoclonal antibody to it) and GLAQ were used as positive and negative controls in each set of 96 pins. The ability of the monoclonal antibody NVS3 to bind to the peptides was tested in an ELISA. Each pin was incubated overnight at 4°C in NVS3 (2 μg of antibody per milliliter). After washing, the pins were incubated for 1 hour at 37°C in goat antibody to mouse IgG (Kirkegaard and Perry, Gaithersburg, Maryland) at a dilution of 1:2000. Optical densities were measured after the pins were incubated in substrate [ABTS (2,2'-azino-di-[3-ethyl-benzthiazoline sulfonate) and hydrogen peroxide] for 30 min.

blocked by preincubating a solution (5 μg/ml) of NVS3 with an equal concentration of the *P. vivax* octapeptide $(AGDR)_2$. Attempts to block NVS3-sporozoite binding with the *P. yoelii* peptide $(QGPGAP)_2$ failed even with concentrations as high as 2 mg/ml (*17*).

Sera from the monkeys that had been passively immunized with NVS3 had high concentrations of antibodies to sporozoites and $(AGDR)_2$ (Table 1). Sera taken from monkeys vaccinated with $NS1_{81}V20$ had similar IFAT titers (range 1:2,560 to 1:10,240). These monkeys were not, however, protected on sporozoite challenge (*7*). In an ELISA, these sera (1:100 and 1:500 final concentrations) reacted with a yeast-produced recombinant protein VIVAX-1 (*18*), which included the same *P. vivax* sequence as $NS1_{81}V20$ (DRA$_{G}^{D}$GQPAG), but these sera from actively immunized monkeys did not react with $(AGDR)_2$ (*18*). When these sera were incubated with increasing amounts of VIVAX-1, all anti–VIVAX-1 activity was removed in a concentration-dependent manner; incubation with $(AGDR)_2$ removed no activity (Fig. 3). Serum samples from these same monkeys were diluted (1:160 to 1:640 depending on individual reactivity to sporozoites) and incubated with VIVAX-1 or $(AGDR)_2$. In IFAT, VIVAX-1 concentrations between 1 and 8 μg/ml eliminated all anti-sporozoite activity but incubation with as much as 2.5 mg/ml of $(AGDR)_2$ removed no activity.

There has been considerable interest in determining if the capacity of sera to inhibit sporozoite invasion of and development in hepatocytes correlates with protection. Sera from the six monkeys vaccinated with $NS1_{81}V20$ (*7*) and from the six passively

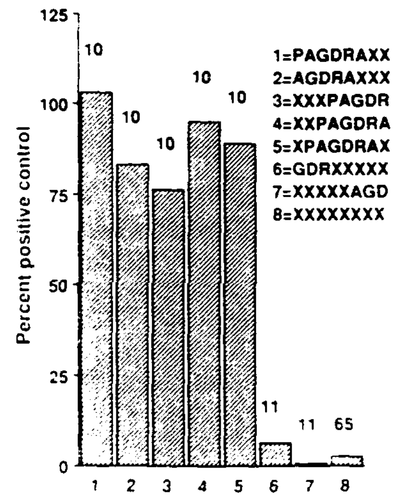

Fig. 2. Antibody-peptide binding is expressed as the percent of the positive control optical density (OD of monoclonal antibody to PLAQ with PLAQ). Bars 1 through 8 represent the mean binding of peptides having the sequences shown in the inset. The number above each bar is the n of that group.

immunized with NVS3 were tested to determine their ability to inhibit sporozoite invasion of and development in *Saimiri* hepatocytes in vitro (*19*). Percent inhibition was 96.5 ± 2.43 (mean ± SEM) (range, 85 to 100%) in the animals immunized with $NS1_{81}V20$ and 98.17 ± 0.60 (range 96 to 100%) in the animals passively immunized with NVS3. These data indicate that, when performed in this manner, sporozoite invasion and development data do not correlate with protection.

We studied the fine specificity of the protective monoclonal antibody NVS3 because we thought that fully characterizing the epitope might explain why polyclonal antibodies induced by the recombinant pro-

tein did not provide protection from sporozoite challenge, whereas apparently equivalent amounts of passively transferred NVS3 did protect. The protective monoclonal antibody recognized only four (AGDR) of the nine (DRA$^{A}_{D}$GQPAG) amino acids comprising the *P. vivax* repeat region. These nine amino acids are the only ones from the CS protein included in the vaccine. A synthetic peptide made of only two copies of AGDR completely inhibited binding of NVS3 to sporozoites and to a recombinant protein (VIVAX-1), which contains a *P. vivax* peptide repeat from the Belem strain. In contrast, sera from monkeys that had been immunized with $NS1_{81}V20$ contained high concentrations of antibodies to sporozoites by IFAT, and the repeat region by ELISA, but had no activity directed against the protective epitope AGDR, as demonstrated by direct ELISA and inhibition studies. It is, of course, possible that antibodies to other epitopes within the nine amino acid *P. vivax* repeat region can protect against sporozoite-induced malaria. However, the fact that the vaccine did not induce antibodies to the only known protective epitope on the *P. vivax* CS protein provides an explanation for the lack of protection.

The monoclonal antibody NVS3 was produced in BALB/c mice; these mice can produce antibodies to AGDR. To further characterize the response to $NS1_{81}V20$, we immunized groups of four BALB/c mice with two doses of 200 μg of $NS1_{81}V20$ or 200 μg of a synthetic peptide, $(AGDR)_6$, conjugated to keyhole limpet hemocyanin (KLH) in complete Freund's adjuvant. Two weeks after the second dose, the animals immunized with $(AGDR)_6$-KLH had excellent antibody responses to $(AGDR)_6$ [1.20 ± 0.028, mean ± SD optical density (OD) of triplicate wells in ELISA at 1:1600 dilution], but a poor response to $NS1_{81}V20$ (0.29 ± 0.010). In contrast, mice immunized with $NS1_{81}V20$ had poor responses to $(AGDR)_6$ (0.19 ± 0.001) and excellent responses to $NS1_{81}V20$ (1.24 ± 0.036). $NS1_{81}V20$ produces poor antibody responses to AGDR in mice and monkeys.

These data demonstrate that it is inappropriate to assume that immunization with a small protein such as $NS1_{81}V20$, which includes a repeating sequence of nine amino acids, will produce antibodies against a single desired epitope such as AGDR. Subsequent vaccine development will undoubtedly require more information regarding the identification of epitopes recognized by protective antibodies and the construction of vaccines that exclude extraneous amino acids. Our data indicate that an appropriate minimal epitope of the *P. vivax* CS protein has been identified. The next challenge is to construct an immunogen that produces antibodies of the desired specificity and to develop an immunization regimen that consistently produces high levels of these antibodies. Questions surrounding immunogen design remain unanswered but the problem of antibody concentration may be solved. In recent studies, humans immunized with a *P. falciparum* CS protein vaccine administered with monophosphoryl lipid A (MPL) and cell wall skeleton of a mycobacterium species as adjuvant or in liposomes with MPL produced concentrations of specific antibodies greater than those found in the sera of monkeys (6 to 18 μg/ml) that received NVS3 in passive transfer (Table 1) (20).

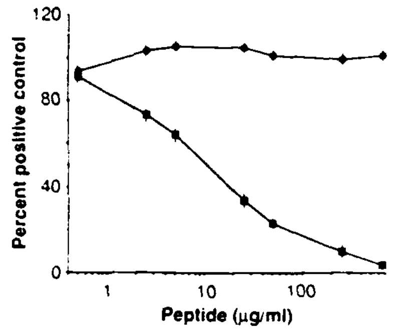

Fig. 3. Serum from six monkeys immunized with $NS1_{81}V20$ given with aluminum hydroxide as the adjuvant were tested in ELISA for activity to VIVAX-1. Secondary antibody was goat antibody to human IgG. Portions of each serum sample (1:250, final concentration) were incubated with varying concentrations of $(AGDR)_2$ (●) or VIVAX-1 (■) to determine if activity to the repeat region of the CS protein can be blocked. Final peptide concentrations are depicted along the *x*-axis.

Table 1. Prepatent periods and NVS3 serum concentrations in monkeys that received 2 mg of monoclonal antibody 1 hour before intravenous challenge with 10^4 *P. vivax* (Salvador I strain) sporozoites. We measured antibody concentrations in IFAT with *P. vivax* sporozoites as antigen and in ELISA using $(AGDR)_2$ as the target antigen. IFAT titers shown are the reciprocals of the last positive dilution. Sera samples (1:100 dilutions) were incubated in $(AGDR)_2$-coated wells. The secondary antibody was horseradish peroxidase–labeled goat antibody to mouse IgG. Optical density values for the serum samples were compared with standard values obtained by measuring the reactivity to $(AGDR)_2$ of known concentrations of NVS3 diluted in equivalent concentrations of *Saimiri* monkey serum. Serum samples taken from each animal immediately before NVS3 injection were all negative for anti-sporozoite activity in IFAT at a dilution of 1:10 and below the sensitivity of the $(AGDR)_2$ ELISA. P, protected in sporozoite challenge; NI, not infected; NT, not tested; Neg, below IFAT and ELISA sensitivity; Try, anti-trypanosomal antibody; *, animals splenectomized on day 6 after challenge, all others on day 7.

Monkey no.	Antibody	Prepatent period	IFAT titer	ELISA NVS3 μg/ml ± SE
SI-74	NVS3	40	6,400	14.3 ± 4.3
SI-162(P)	NVS3	NI	12,800	18.4 ± 7
SI-218	NVS3	31	3,200	4.3 ± 1.5
SI-250(P)	NVS3	NI	6,400	18.5 ± 2.5
SI-251(P)	NVS3	NI	6,400	7.2 ± 1.8
SI-312(P)	NVS3	NI	6,400	5.9 ± 1.8
SI-323	Try	NI	NT	NT
SI-319	Try	23	Neg	Neg
SI-330	Try	15	NT	NT
SI-316	Try	24	Neg	Neg
SI-321	Try	23	Neg	Neg
SI-328	Try	18	NT	NT
SI-311*	None	29	NT	NT
SI-238*	None	18	NT	NT
SI-320	None	17	NT	NT
SI-45*	None	30	NT	NT
SI-249	None	19	NT	NT
SI-174	None	20	NT	NT
SI-289	None	21	NT	NT
SI-101*	None	17	NT	NT
SI-300	None	19	NT	NT

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8. *Saimiri sciureus boliviensis* monkeys of Bolivian origin were used in the passive transfer study after being quarantined for a 1 month conditioning period. Monkeys were weighed, tested for tuberculosis, and examined for concurrent parasitic infections of the intestine and blood.
9. Female, 6- to 8-week-old BALB/c ByJ mice (Jackson Laboratory, Bar Harbor, ME) were used in the production of monoclonal antibodies as previously described [Y. Charoenvit, M. F. Leef, L. F. Yuan, M. Sedegah, R. L. Beaudoin, *Infect. Immun.* **55**, 604 (1987)]. *Plasmodium vivax* sporozoites of the Vietnam (ONG/CDC) and North Korean (NK) strains and a Colombian isolate were used as immunogen. Species and stage specificities were determined in an IFAT against sporozoites and blood stage parasites

from *P. vivax*, *P. falciparum*, *P. berghei*, *P. yoelii*, and *P. gallinaceum*. Reactivity to different strains of *P. vivax* (North Korean, Salvador 1, Colombian, and Thai) was also measured. The monoclonal antibody selected for passive transfer was designated NVS3 (Navy Vivax Sporozoite 3) and purified by affinity-column chromatography with staphylococcal protein A coupled to Sepharose 4B [H. Hjelm and J. Sjoquist, in *Immunoadsorbants in Protein Purification*, E. Ruoslahti, Ed. (University Park Press, Baltimore, MD, 1976), p. 51]. NVS3 was selected because it is species- and stage-specific and had the greatest activity in the IFAT against *P. vivax* sporozoites.

10. Sporozoites of the Salvador I strain were reared in *Anopheles stephensi* mosquitoes by membrane feeding the mosquitoes on gametocytemic chimpanzee blood [W. E. Collins, H. M. McClure, R. B. Swenson, P. C. Mehaffey, J. C. Skinner, *Am. J. Trop. Med. Hyg.* **35**, 56 (1986)]. Sixteen days after feeding, the sporozoites were dissected from the salivary glands of the infected mosquitoes for use in the challenge studies. On the basis of initial experiments, a dose of 2 mg of NVS3 per monkey was selected for injection intravenously into six *Saimiri* monkeys. An IgG3 monoclonal antibody directed against *Trypanosoma brucei rhodesiense* [T. Hall and K. Esser, *J. Immunol.* **132**, 2059 (1984)] was inoculated into another six monkeys to serve as an unrelated antibody control group. Nine other monkeys served as uninjected controls. One hour after antibody transfer, 10^4 *P. vivax* sporozoites in normal saline and 10% normal *Saimiri* monkey serum were injected into all monkeys. Serum samples were collected before antibody inoculation and 1 hour later (immediately before sporozoite challenge). All animals were splenectomized 6 to 7 days after sporozoite inoculation. Beginning 14 days after sporozoite inoculation and continuing through day 56, Giemsa-stained thick and thin blood films were prepared daily. Parasitemias were quantified and recorded per cubic millimeter of blood.
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15. R. Rosenberg *et al.*, *Science* **245**, 973 (1989).
16. The eight-residue peptide $(AGDR)_2$ was synthesized by the stepwise solid-phase method [R. B. Merrifield, *J. Am. Chem. Soc.* **85**, 2149 (1963)]. Pam-*t*-Boc-L-arginine (Tos) resin (0.5 mmol) was used as the starting point of the synthesis. The protected peptide resin was deprotected by hydrogen fluoride–*p*-cresol (9:1, v/v) for 1 hour at 0°C.
17. NVS3 activity was measured in the serum of the monkeys that received intravenous NVS3 before sporozoite challenge. Twofold serial dilutions of sera were used in an IFAT with air-dried *P. vivax* sporozoites as the target antigen. To determine if NVS3 reacts with epitopes other than AGDR on sporozoites, aliquots of NVS3 at a concentration of 2.5 μg/ml were incubated with varying amounts of the *P. vivax* peptide $(AGDR)_2$ or the unrelated peptide $(QGPGAP)_2$, a peptide from the repeat region of *P. vivax* CS protein. The antibody-peptide mixtures were then incubated with *P. vivax* sporozoites and evaluated by IFAT to measure the ability of $(AGDR)_2$ to block the binding of NVS3 to sporozoites.
18. VIVAX-1 is a recombinant protein containing approximately 60% of the entire CS protein from the Belem strain of *P. vivax*. It contains the repeat regions $(DRAAGQPAG)_{20}$ [P. J. Barr *et al.*, *J. Exp. Med.* **165**, 1160 (1987)]. $NS1_{81}V20$ vaccine is a fusion protein from *E. coli* that contains 20 copies of the nonapeptide repeat present in the repeat region of the CS protein and 81 amino acids derived from the nonstructural protein gene of influenza A [D. M. Gordon *et al.*, *Am. J. Trop. Med. Hyg.* **42**, 527 (1990)].
19. *Saimiri* monkey liver fragments were dissociated by collagenase perfusion and plated in 35-mm petri dishes. Equal volumes of serum and sporozoite suspension were mixed and incubated at room temperature for 15 min. The serum-sporozoite mixtures were exposed to the hepatocytes for 2 hours then washed. Seven days after exposure, the monolayers were fixed and schizonts counted microscopically [P. Millet *et al.*, *Am. J. Trop. Med. Hyg.* **38**, 340 (1988)].
20. L. S. Rickman *et al.*, *Clin. Res.* **38**, 352A (1990); C. R. Alving *et al.*, in preparation.
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Ability of the c-*mos* Product to Associate with and Phosphorylate Tubulin

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The *mos* proto-oncogene product, pp39^{mos}, is a protein kinase and has been equated with cytostatic factor (CSF), an activity in unfertilized eggs that is thought to be responsible for the arrest of meiosis at metaphase II. The biochemical properties and potential substrates of pp39^{mos} were examined in unfertilized eggs and in transformed cells in order to study how the protein functions both as CSF and in transformation. The pp39^{mos} protein associated with polymers under conditions that favor tubulin oligomerization and was present in an approximately 500-kilodalton "core" complex under conditions that favor depolymerization. β-Tubulin was preferentially coprecipitated in pp39^{mos} immunoprecipitates and was the major phosphorylated product in a pp39^{mos}-dependent immune complex kinase assay. Immunofluorescence analysis of NIH 3T3 cells transformed with *Xenopus* c-*mos* showed that pp39^{mos} colocalizes with tubulin in the spindle during metaphase and in the midbody and asters during telophase. Disruption of microtubules with nocodazole affected tubulin and pp39^{mos} organization in the same way. It therefore appears that pp39^{mos} is a tubulin-associated protein kinase and may thus participate in the modification of microtubules and contribute to the formation of the spindle. This activity expressed during interphase in somatic cells may be responsible for the transforming activity of pp39^{mos}.

THE PROTO-ONCOGENE C-*mos* IS EXpressed at high levels in the germ cells of vertebrates (*1, 2*). In *Xenopus* and mouse oocytes, the *mos*-encoded protein, pp39^{mos}, is expressed during oocyte maturation and is required for maturation before and after germinal vesicle breakdown (GVBD) (*2–5*). Injection of *mos* RNA into fully grown *Xenopus* oocytes can induce both GVBD and maturation promoting factor (MPF) (*3*), which is composed of cyclin and p34^{cdc2} (*6*). MPF activation is correlated with GVBD, chromosome condensation, and spindle formation (*7–9*). A second activity present in mature oocytes, cytostatic factor (CSF), is thought to be responsible for the arrest of unfertilized eggs at meiotic metaphase II (*10*). Thus, CSF injected into blastomeres of cleaving embryos arrests cell cleavage at metaphase of mitosis (*10–12*), which is a major control point of the cell cycle (*9*). MPF is present at large concentrations in metaphase II oocytes, and it has been proposed that CSF stabilizes MPF (*7, 9, 13, 14*). Recently, pp39^{mos} has been shown to be the active component in CSF (*13*). It is possible to isolate pp39^{mos} from cytosolic extracts by high-speed centrifugation (*13*), which suggests that it is present in a large complex. In addition, the large size of the mitotic spindle in CSF-arrested blastomeres (*11*) and the ability of taxol—a plant diterpene antineoplastic agent that binds to and stabilizes microtubules (*15, 16*)—to arrest cleaving embryos at metaphase (*16*) suggested to us that pp39^{mos} might be associated with tubulin.

We subjected a CSF cytosolic extract pre-

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